1431-Pos Board B161
Exploring the Energy Landscape of RNA: A Direct Evaluation of the Counterion Mediated Free Energy
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Functional non-coding RNA molecules must assume a specific tertiary structure in order to properly catalyze important reactions in vivo. Due to the intrinsic negative charge of RNA, this folding process is inherently dependent on the interplay between the RNA molecule itself and its surrounding positive counterions which neutralize the overall charge and allow for stabilization of the RNA tertiary structure. We present here a novel algorithmic approach to calculating the divalent-ion mediated free energy of an RNA conformation based on established theory that considers the ions explicitly. By efficiently searching the local energy landscape, the free energy of the system can be evaluated quickly and directly. The speed and accuracy of this direct approach allows for simple integration into preexisting all-atom RNA simulation with which we demonstrate ion-mediated effects on the P4-P6 domain of the Tetrahymena Group I Intron.

1432-Pos Board B162
L-Proline Destabilization of RNA Duplexes is Temperature Dependent
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L-proline is an osmoprotectant and nonessential amino acid that destabilizes nucleic acid secondary and tertiary structures and, at high concentrations, denatures nucleic acids. In this work the interactions between L-proline and the functional groups on the solvent accessible nucleic acid surface area that would be exposed by unfolding (ΔASA) of eleven RNA dodecamer duplexes with guanine-cytosine (GC) content ranging from 17-100% were quantified using thermal and titration denaturation monitored by uv-absorbance. L-proline destabilized GC-rich duplexes to a greater degree during thermal denaturation than low GC-content duplexes due to greater accumulation of L-proline in the vicinity of the RNA ΔASA. This accumulation was temperature dependent; L-proline-RNA interactions quantified using titration denaturation at 25 C were nearly independent of GC-content. Thus, the greater destabilization of RNA GC-rich duplexes observed during thermal denaturation was due to greater L-proline interactions with the RNA ΔASA at the higher transition temperatures of GC-rich duplexes. Our results suggest L-proline interactions are entropically-driven, potentially due to dehydration of L-proline when interacting with the RNA ΔASA. In addition, L-proline destabilized the RNA duplexes to a greater degree than glycine betaine and urea. This suggests glycine betaine, L-proline, and urea have the potential to form a triad of cosolutes to probe changes in solvent accessible surface area during biochemical reactions.

1433-Pos Board B163
Targeting the Hepatitis C Virus with PNAs
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The liver specific microRNA-122 (miR-122) has been shown to facilitate the replication and/or translation of the Hepatitis C virus (HCV). Although the exact role played by miR-122 in this process is not fully understood, it has been shown that one of the functions of miR-122 is to stabilize the HCV RNA genome. It has also been shown that miR-122 is a valid antiviral target, as locked nucleic acids (LNAs) developed against miR-122 abolished HCV replication. However, miR-122 has numerous other functions in the hepatic cell, which will also be affected by LNAs. In this study, we adopted a different approach by designing peptide nucleic acids (PNAs) against the highly conserved miR-122 binding sites within the HCV genome, and tested their antiviral properties. We demonstrated the ability of the PNA to bind specifically the 5' UTR of the HCV genome, with a low nanomolar dissociation constant. Moreover, we showed that the PNA is able to invade the HCV 5'-UTR - miR-122 complex displacing miR-122, and thus preventing it from exerting its beneficial function upon HCV replication and/or translation.

1434-Pos Board B164
Time-Resolved and Dynamic Studies of Riboswitches
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Riboswitches are a class of regulatory RNA molecules widely distributed mostly across prokaryotic organisms. The discovery of the first riboswitches, by the Breaker lab and others, opened the door to a previously unimagined layer of genetic regulation. The majority of riboswitches undergo large scale conformational rearrangements upon binding target metabolites in their aptamer domain. Riboswitches are found predominantly in the 5' untranslated region of a number of genes. As a nascent strand is being transcribed by an RNA polymerase, the switch can fold to either occlude the expression platform or to expose it. The former leads to termination of the transcript, while the latter leads to gene expression. It is hypothesized that the concentration of the riboswitch's target metabolite governs gene expression. Thus, if the switch has bound its target metabolite prior to moving past the critical terminator stem, gene expression is regulated. Conversely, if the polymerase moves past the terminator stem before binding the target metabolite, the riboswitch and metabolite will not have time to reach equilibrium before an on/off decision is made. Accordingly, the mechanism of regulation may be highly dependent on the kinetic on-rate. For these reasons, understanding the dynamics of the riboswitch both on a global and local level is critical to determining the molecular mechanism by which riboswitches regulate gene expression. NMR T1, T2, NOE, cross-correlation, and dispersion experiments would be presented to delineate the site-specific dynamics involved in riboswitch function. These local dynamics measurements are combined with other biophysical techniques that we use to track global structural changes on a wide range of time-scales. These studies would likely provide a glimpse of the conformational switching necessary for riboswitch function.

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RNA Structural Rearrangements during Reverse Transcription Initiation in HIV
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Reverse transcription is the first step in the replication of the Human Immunodeficiency Virus (HIV) and is a target of multiple therapies. The initiation phase of reverse transcription in human immunodeficiency virus (HIV) is the slowest and least processive step of the reverse transcription process; however, premature initiation of reverse transcription leads to failed infection. During this phase, several structures between the viral genomic RNA and tRNA(Lys3) primer are formed and broken as reverse transcriptase transcribes viral genomic RNA into negative sense DNA. Using NMR spectroscopy, X-Ray crystallography, single molecule FRET spectroscopy, and biochemical methods, we are characterizing these RNA structural rearrangements in order to understand their role in regulating the initiation of reverse transcription in HIV.

1436-Pos Board B166
Exploring the Genotype/Phenotype Landscape of Self-Assembling Modules in RNA
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GNRA tetraloops (N for base, R for purine) in tertiary interaction with distant helices or receptors are among the most widespread self-assembling modules identified in functional RNAs in nature. A remarkable example is the GAAA/11nt receptor motif, which displays a strong binding affinity and selectivity for its cognate tetraloop. However, *in vitro* evolution has yielded novel receptors with binding affinities and selectivity for GNRA tetraloops similar to that of the GAAA/11nt receptor, raising the question why only a limited subset of these interactions occurs in nature. It has been proposed that the natural evolution of GNRA receptors may incur a selection pressure to favor sequences that display a high degree of mutational robustness, meaning that single point mutations to their sequence do not significantly alter their self-assembly function. To this end, we have performed a comprehensive mutational analysis of the 11nt motif and two artificial receptors isolated by *in vitro* selection and shown to recognize their cognate tetraloops with binding affinity comparable to that of the GAAA-11nt interaction. Using a tectoRNA system, a comprehensive genetic/phenotypic landscape was derived for each of these interactions by screening their variants for the ability to bind GNRA tetraloops. The results indicate that both natural and artificial receptors maintain good binding affinity for their cognate tetraloop (GAAA-11nt; GUAA-R5.58; R1-GGAA) upon single point mutation. However, the artificial receptor R5.58 displays a capacity to change its phenotype in terms of its affinity and selectivity toward a particular GNRA tetraloop, which may indicate a greater degree of plasticity and evolvability through single point mutation. Artificially selected receptors may once have occurred in nature, as evolutionary intermediates on the way to optimal solutions, like the 11nt motif. Further experiments are planned to validate this hypothesis.